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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/593,016	09/14/2006	Thaddeus C. George	BIOL0123	5733

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EXAMINER	
HEIDEMANN, JASON E	

ART UNIT	PAPER NUMBER
2624	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/593,016	GEORGE ET AL.	
	Examiner	Art Unit	
	JASON HEIDEMANN	2624	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 May 2011*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-24,29-31,33,34,36,37,39,40 and 45-48* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-24, 29-31, 33, 34, 36, 37, 39, 40, and 45-48* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *12 June 2008* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *04/25/2011, 06/02/2011, 06/20/2011*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

General Information Matter

1. Applicant filed Amendment on 05/02/2011 for application 10/593016 amending claims16, 23, 24, 29, 30, 31, 33, 34, 36, 37, 39 and 40, and canceling 27, 28, 32, 35, 38, and 41-44. Currently, Claims 1-24, 29-31, 33, 34, 36, 37, 39, 40, and 45-48 are pending

Response to Amendment

The amendment received 05/02/2011 has been entered and considered in full.

Response to Arguments

35 USC § 112

Claims 30-44 were rejected under 35 USC § 112 a as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicant has since amended claims (30-31, 33-34, 36-37, 39-40) and cancelled claims (32, 35, 38, 41-44) to remedy the issues noted in the previous action; therefore, the previously outstanding grounds of rejection under 35 USC § 112 ¶2 are hereby withdrawn.

35 USC § 102/103

- Claims 27 and 28 were rejected under 35 U.S.C. 102(b) as being anticipated over
- Elling Claim 27 was further rejected and Claim 29 was rejected under 35 U.S.C. 102(e) as being anticipated over Ortyn.

- Claims 1-23 were rejected under 35 U.S.C. 103(a) as being unpatentable over Ortyn in view of Young.
- Claim 24 was rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Ortyn and Young as applied above and further in view of Fraatz
- Claims 30-41 and 44 were rejected under 35 U.S.C. 103(a) as being unpatentable over Ortyn in view of Nicoletti
- Claims 42 and 43 were rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Ortyn and Nicoletti, further in view of Vitale
- Claim 28 was further rejected under 35 U.S.C. 103(a) as being unpatentable over Ortyn as applied above in view of Fraatz.

Applicant's arguments, that the Ortyn is not prior art is persuasive. Therefore, the rejections using Ortyn (20040021868) has been withdrawn. However, upon further consideration, new grounds of rejection have been made in view of the previously presented disclosures of:

- Ortyn et al. ("Ortyn") [US PGPub #2002/0071121]
- Basiji et al. ("Basiji") [US Patent #, 6211955]

Applicant argues that there invention represents a non-obvious modification of Nicoletti's disclosure (Applicant's remarks page 15). However, Applicant admits on page 16, that the imaging system in applicant's invention used to classify the cells is well known (why the prior art disclosures of Ortyn and Basiji are reintroduced), and further calculating the features used to identify the cells is also well known (e.g. spatial frequency) (Applicant's remarks page 16). Applicant's argues that selecting spatial frequency for comparison to identify the viability of a

cell represents the non-obvious modification. However, the disclosure of both Ortyn'121 and Basiji'955 suggests that these features could be used in cell analysis. Thus the system could be modified to use Nicoletti's cytometry methods and teachings "Apoptotic cells display a very specific pattern of morphological changes at the light, electron and fluorescence microscope and this should be the deciding factor when ambiguity arises regarding the mechanism of cell death" to suggest to one skilled in the art to identify cells using those localized cell features (spatial frequency). Further, Young discloses using the well known template matching classifier for classifying cells. Thus one skilled and creative in the art would have recognized using a template matching classifier on spatial frequency features to assist (or enhance) the performance of cell classification.

Applicant's additionally argues on page 16, that why the imaging system used in the invention is well known and that none of the prior arts disclose which of the many disclosed metric could be used to achieve a cell classifier to identify the viability of a cell. Examiner respectively disagrees, the list of features is finite and it is routine in the art for a researcher to assess which feature(s) would assist in classification of a cell. Thus, given the well known methods for classification, the available finite features, it would have been obvious to try using spatial frequency components of a cell for viability classification with a reasonable expectation of success.

The test for obviousness is what the combined teachings of the references would have suggested to one of ordinary skill in the art. *See In re Kahn*, 441 F.3d at 987-88; *In re Young*, 927 F.2d 588, 591 (Fed. Cir. 1991); and *In re Keller*, 642 F.2d 413, 425 (CCPA 1981). Moreover, in evaluating such references, it is proper to take into account not only the specific teachings of the

references but also the inferences which one skilled in the art would reasonably be expected to draw therefrom. *In re Preda*, 401 F.2d 825, 826 (CCPA 1968).

Section '103 forbids issuance of a patent when "the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains." *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1734 (2007).

In *KSR*, the Supreme Court emphasized "the need for caution in granting a patent based on the combination of elements found in the prior art," and discussed circumstances in which a patent might be determined to be obvious. *KSR*, 127 S. Ct. at 1739 (citing *Graham v. John Deere Co.*, 383 U.S. 1, 12 (1966)). The Court reaffirmed principles based on its precedent that "[t]he combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." *Id.* The operative question in this "functional approach" is thus "whether the improvement is more than the predictable use of prior art elements according to their established functions." *Id.* at 1740.

The Examiner believes that all the arguments of the Applicant(s) have been properly addressed and explained.

Priority

This application claims benefit of a National Stage Application No.PCT/US05/08870, filed 03/16/2005. This application claims benefit of an earlier filing date under 35 U.S.C. 119(e) of U.S. Provisional Application 60/553502, filed 16 March 2004.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on 06/20/2011, 06/02/2011, and 04/25/2011 are in compliance with the provisions of 37 C.F.R. § 1.97. Accordingly, the examiner has considered all references cited in the submitted IDS(s).

Claim Objections

Claim 45 and 48 are identical. Appropriate correction is required.

Examiner's Note

Examiner has cited particular columns and line numbers or figures in the references as applied to the claims below for the convenience of the applicant. Although the specified citations are representative of the teachings in the art and are applied to the specific limitations within the individual claim, other passages and figures may apply as well. It is respectfully requested from the applicant, in preparing the responses, to fully consider the references in entirety as potentially

teaching all or part of the claimed invention, as well as the context of the passage as taught by the prior art or disclosed by the examiner.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 45-48 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 45 and 48, each recite the limitation of "wherein the spatial frequency content of the side scatter image is calculated by computing a standard deviation of individual pixel intensities within the image", however it is unclear which image is the image, since Claim 1 recites two images a side scatter image of a known cell and a side scatter image of a specific cell.

Claims 46 recites the limitation of "wherein the spatial frequency content of the brightfield image is calculated by computing a standard deviation of individual pixel intensities within the image", however it is unclear which image is the image, since Claim 8 recites two images a brightfield image of a known cell and a brightfield image of a specific cell.

Claims 47 recites the limitation of "wherein the spatial frequency content of the brightfield image is calculated by computing a standard deviation of individual pixel intensities

within the image", however it is unclear which image is the image, since Claim 16 recites two images an image of a known cell and an image of a marked specific cell.

Claim Rejections - 35 USC § 103

1. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

A.) Claims 1-7, 29-31, 33, 34, 36, 37, 39 and 40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nicoletti et al. ("Nicoletti") ["Common Methods for Measuring Apoptotic Cell Death by Flow Cytometry", 1997, The Purdue Cytometry CD-ROM Volume 3, Purdue University, West Lafayette, ISBN 1-890473-02-2] in view of Basiji et al. ("Basiji") [US Patent #, 6211955] and further in view of Young et al. ("Young") ["Towards automatic cell identification in DIC microscopy", November 1998, Journal of Microscopy, Vol. 192, Pt 2, pp. 186–193]

As to Claim 1, Nicoletti outlines the problem to be solved (identifying a specific cell), and discloses a method for identifying a specific cell, to enable a determination to be made as to whether the specific cell corresponds to a known cell type, wherein the known cell type relates to the viability of the cell, (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, distinguish between late state apoptotic cells, necrotic cells, and viable cells***), comprising: providing content data from a side scatter image of the known cell type, and comparing the content to determine the cell type (***Nicoletti, Fig. 2, see page 2, section a., Physical parameters of apoptotic cells ¶1-2, page 3, section b. DNA content analysis ¶1, page 4, ¶1 - 2, teaches common methods that exploit physical parameters (cell size, granularity, cell density) and DNA content analysis (stainability) of cells to determine their viability, using combination of light scattered in the forward direction (brightfield image) and a lateral direction (side scatter image)***). Further, Nicoletti discloses regardless of the flow cytometry technique used to measure apoptosis, in most situations the type of cell death should be confirmed by direct microscope inspection. Apoptotic cells display a very specific pattern of morphological changes at the light, electron and fluorescence microscope and this should be the deciding factor when ambiguity arises regarding the mechanism of cell death (***Nicoletti, Fig. 2, see page 2, section a., Physical parameters of apoptotic cells ¶1-2, page 3, section b. DNA content analysis ¶1,, page 4, ¶1 - 2, teaches common methods that exploit physical parameters (cell size, granularity, cell density) and DNA content analysis (stainability) of cells to determine their viability***). However Nicoletti silent to collecting spatial frequency of a specific cell, and comparing spatial frequency of a know cell type to the collected spatial frequency of the specific cell to determine if specific cell corresponds to the known cell type.

Enter Basiji, who teaches a system that performs a method for collecting features for examining a specific cell, comprising:

directing incident light at a cell (***Basiji, US Patent 6211955, Abstract, Column 6, Lines 15-16, a light source is diposed to provide an incident light that illuminates the object (cell)***), using a detector to obtain a side scatter image (***Basiji, 6211955, Fig. 5, Fig.6, Abstract, Column 6, Lines 15-26, Lines 43-54, a detector is used to collect the scatter image of the object, the detectors are perpendicular to the light beam (Side scatter)***). Collecting spatial frequency content data of an individual cell from a side scatter image (***Basiji, 6211955, Column 8, Lines 24-47, Column 17, Lines 27-31***). Furthermore, Basiji teaches using spatial frequency content to be used in cell analysis, and further suggests that a cell can be identified using the morphological parameters (spatial frequency content) (***Basiji, 6211955, Column 8, Lines 24-47, Column 17, Lines 27-31***).

One skill in the art would have been motivated to combine the teachings of Nicoletti to the apparatus of Basiji. Basiji's appartus is an improvement over a basic flow cytomerty, as it offers considerable advantages over systems employed for cell and particle analysis in the prior art, specifically the ability to combine an optical dispersion system with a TDI detector that produces an output signal in response to the images of cells and other objects that are directed on the TDI detector (***Basiji, 6211955, Column 8, Lines 13-18***). Basiji's apparatus allows imaging of multiple objects on the TDI detector at the same time, and each object in the image can be spectrally decomposed to discriminate object features by absorption, scatter, reflection or probe emissions using a common TDI detector for analysis (***Basiji, 6211955, Column 8, Lines 18-23***). Basiji's apparatus solves the caveat disclosed by Nicolette "most situations the type of cell death

should be confirmed by direct microscope inspection", as the TDI detector can replace the requirement for direct microscope inspection, as the TDI detector can achieve a desired resolution (optical magnification).

However, the combination of Nicolette and Basiji are silent to a determining as to whether the specific cell corresponds to a known cell type, comprising the steps of: providing spatial frequency content data from a side scatter image of the known cell type; and comparing the spatial frequency content of the side scatter image of the specific cell to the spatial frequency content data of the side scatter image of the known cell type to determine if the specific cell corresponds to the known cell type.

Enter Young, who discloses a general method for cell identification based on template matching (cell comparison), where the templates are constructed from a known cell types (***Young, abstract, page 192, section 4. discussion***). Further, Young motivates this method by demonstrated its ability to automatically identify and measure individual cells in clusters (***Young, abstract***). Young's "Template matching" is a method for automating the identification and measuring of cells in microscope image, where correspondences are found between a template sub-image and the full image, based on a goodness-of-fit statistic evaluated at all possible positions, where Young utilizes FFTs in order to reduce computer time to an acceptable level (***Young, abstract, page 187, left col., paragraph 3***).

One skilled in the art would have recognized that this technique of template matching, the comparing a known cell's features to a specific cell's feature in order to classify the cell, would have been obvious to attempt using the feature of spatial frequency of a known cell to classify other cells, due to it's well known success in classification based on features. The modification

to the combination of Nicolette and Basiji, could be accomplished by including a template matching algorithm, which allows the comparison of a template, previous collected data of a known type (template), to newly acquired image data to identify the individual cell. Further a person of ordinary skill in the art would have recognized the compatibility of template matching with the method of the combination of Nicolette and Basiji. The combination has a reasonable expectation of success in that the modifications can be made using conventional and well known engineering and/or programming techniques, the template matching as taught by Young is not altered and continues to perform the same function as separately, and the resultant combination produces the highly predictable result of "comparing the spatial frequency content of the side scatter image of the specific cell to the spatial frequency content data of the side scatter image of the known cell type to determine if the specific cell corresponds to the known cell type."

As to claim 2, the combination of Nicoletti, Basiji and Young teach the method of claim 1 wherein there is relative motion between the specific cell and the detector (***Basiji, 6211955, Column 2, Column 3, Lines 50-62, Line 49-50, Column 4, Lines 6-28, the detector captures the velocity (relative motion) between the cells and the detector***).

As to claim 3, the combination of Nicoletti, Basiji and Young teach the combination of Nicoletti, Basiji and Young teach the method of claim 1 wherein the specific cell identified is contained within a heterogeneous cell population, and side scatter image data is collected for the heterogeneous cell population (***Basiji, 6211955, Abstract, Column 3, Line 62-67, Column 4, Lines 1-6***) (***Nicoletti, see page 3, section a. Physical parameters of apoptotic cells, ¶4, "to identify the phenotype of apoptotic cells in a heterogeneous cell population"***).

As to claim 4, the combination of Nicoletti, Basiji and Young teach the method of claim 1 wherein the specific cell identified is an apoptotic cell (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, distinguish "apoptotic cells"***).

As to claim 5, the combination of Nicoletti, Basiji and Young teach the method of claim 4 wherein the apoptotic cell is an early stage apoptotic cell ***or a late stage apoptotic cell*** (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, distinguish between late state apoptotic cells***)

As to claim 6, the combination of Nicoletti, Basiji and Young teach the method of claim 1 wherein the specific cell identified is a necrotic cell (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, distinguish "necrotic cells"***).

As to claim 7, the combination of Nicoletti, Basiji and Young teach the method of claim 1 wherein the specific cell identified is at least one of an apoptotic cell and a necrotic cell (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, distinguish between late state apoptotic cells, necrotic cells, and viable cells***).

As to Claim 29, Nicoletti discloses a method for of classifying a specific cell as one of the following four types of cells, a viable cell (***Nicoletti, page 4, ¶2-3, page 5, ¶1. "Whereas the DNA content of unfixed apoptotic cells is unchanged compared to normal (viable) cells", "the difference in the DNA fluorescence of normal (viable) and apoptotic cells is minimal or undetectable" page3, ¶1-2, thus in view of the reduced stainability of apoptotic cells, one concludes the viable cells also have a reduced stainability(impermeable), further Nicoletti discloses comparing measurable morphological and physical properties (cells can be***

distinguished based on cell sizes see cells undergoing apoptosis exhibit cell shrinkage, condensation of chromatin and cytoplasm and necrotic death of a cell is characterized by a reduction in both FSC and SSC (probably due to a rupture of plasma membrane and leakage of the cell's content), so a viable cell what have a larger cellular area than say cells undergoing apoptosis), a necrotic cell (***Nicoletti, page 2, ¶2-3, necrotic cells take on stain further the teachings discloses measurable morphological and physical properties, cellular size would increase in view of rupture of plasma membrane and leakage of cell content***), an early apoptotic cell in which a cellular membrane of the cell is still intact (***Nicoletti, page 2, ¶1, page 3, ¶4, page 4, ¶1-2, one would expect to determine an early stage of apoptosis based on the teachings of reduced stainability of apoptotic cells (cellular membrane of the cell is still intact))is the direct consequence of partial loss of DNA due to the activation of endogenous nucleases and diffusion of low-molecular weight DNA outside the cells", further the teachings discloses measurable morphological and physical properties (cell shrinkage, condensation of chromatin and cytoplasm)***), and a late apoptotic cell in which the cellular membrane of the cell is not intact (***Nicoletti, page 2, ¶1-3, given the emerging secondary necrosis of later stages of apoptosis, one skilled in the art would expect to find traces of stained cellular DNA (cellular membrane of the cell is NOT intact), given that necrotic cells take on stain (In later stages of apoptosis the intensity of both FSC and SSC decreases due to the emerging secondary necrosis), further the teachings discloses measurable morphological and physical properties (cell shrinkage, condensation of chromatin and cytoplasm)***), using only a single nuclear marker (***Nicoletti, page 3-6, a method using a single nuclear stain to distinguish between late state apoptotic cells, necrotic cells, and viable cells, and teachings (page 6) of using morphologic***

features to distinguish early stage apoptotic cells from viable cells, as they prevent the stain from crossing the cellular membrane – requires a microscope evaluation) and image data from the cell, the method comprising the steps of:

exposing the specific cell to only a single nuclear marker that will bind to DNA in a nucleus of the cell in the event that the cellular membrane of the cell is not intact (***Nicoletti, Fig. 3, see page 4, ¶4, page 5, ¶1 teaches staining with PI or DAPI stain help identify a cell, see page 2, ¶1-3, necrotic cells take on stain and later stages of apoptosis which take on stain***);

collecting image data from the specific cell (***Nicoletti, Fig. 2, see pages 2, ¶2, see collecting light scatter in the forward direction, and in the lateral direction***);

analyzing the image data to classify the cell as one of a viable cell, a necrotic cell, an early apoptotic cell, and a late apoptotic cell (***Nicoletti, Fig. 2, see pages 2-3, section a, which outline ways to distinguish the different cells***). Further, Nicoletti discloses regardless of the flow cytometry technique used to measure apoptosis, in most situations the type of cell death should be confirmed by direct microscope inspection. Apoptotic cells display a very specific pattern of morphological changes at the light, electron and fluorescence microscope and this should be the deciding factor when ambiguity arises regarding the mechanism of cell death (***Nicoletti, Fig. 2, see page 2, section a., Physical parameters of apoptotic cells ¶1-2, page 3, section b. DNA content analysis ¶1,, page 4, ¶1 - 2, teaches common methods that exploit physical parameters (cell size, granularity, cell density) and DNA content analysis (stainability) of cells to determine their viability***). However Nicoletti silent to using the image data of the specific cell to determine a spatial frequency content of a side scatter image of the specific cell and analyzing

the image data of the specific cell and the spatial frequency content of the side scatter image of the specific cell to classify the cell as one of a viable cell, a necrotic cell, an early apoptotic cell, and a late apoptotic cell.

Enter Basiji, who teaches a system that performs a method for collecting features for examining a specific cell, comprising: exposing the specific cell to a single nuclear marker (***Basiji, US Patent 6211955, Abstract, Column 12, Lines 36-49, dislcoses collecting images of cells that are stained with a flouresecnet stain***) using the image data of the specific cell to determine a spatial frequency content of a side scatter image of the specific cell (***Basiji, US Patent 6211955, Fig. 5, Fig.6, Abstract, Column 6, Lines 15-26, Lines 43-54,, a light source is diposed to provide an incident light that illuminates the object (cell) and a detector is used to collect the scatter image of the object, the detectors are perpendicular to the light beam (Side scatter), futher Column 8, Lines 24-47, Column 17, Lines 27-31 dislcoses collecting the spatial freuqneyc content data of the side scatter of an individual cell***). Furthermore, Basiji teaches using spatial frequency content to be used in cell analysis, and further suggests that a cell can be identified using the morphological parameters (spatial frequency content) (***Basiji, 6211955, Column 8, Lines 24-47, Column 17, Lines 27-31***).

One skill in the art would have been motivated to combine the teachings of Nicoletti to the apparatus of Basiji. Basiji's appartus is an improvement over a basic flow cytomerty, as it offers considerable advantages over systems employed for cell and particle analysis in the prior art, specifically the ability to combine an optical dispersion system with a TDI detector that produces an output signal in response to the images of cells and other objects that are directed on the TDI detector (***Basiji, 6211955, Column 8, Lines 13-18***). Basiji's apparatus allows imaging of

multiple objects on the TDI detector at the same time, and each object in the image can be spectrally decomposed to discriminate object features by absorption, scatter, reflection or probe emissions using a common TDI detector for analysis (***Basiji, 6211955, Column 8, Lines 18-23***). Basiji's apparatus solves the caveat disclosed by Nicolette "most situations the type of cell death should be confirmed by direct microscope inspection", as the TDI detector can replace the requirement for direct microscope inspection, as the TDI detector can achieve a desired resolution (optical magnification).

However, the combination of Nicolette and Basiji are silent to how to use the spatial freuquency for assisting in classificaiton of the cell.

Enter Young, who discloses a general method for cell identification based on template matching (cell comparison), where the templates are constructed from a known cell types (***Young, abstract, page 192, section 4. discussion***). Further, Young motivates this method by demonstrated its ability to automatically identify and measure individual cells in clusters (***Young, abstract***). Young's "Template matching" is a method for automating the identification and measuring of cells in microscope image, where correspondences are found between a template sub-image and the full image, based on a goodness-of-fit statistic evaluated at all possible positions, where Young utilizes FFTs in order to reduce computer time to an acceptable level (***Young, abstract, page 187, left col., paragraph 3***).

One skilled in the art would have recognized that this technique of template matching, the comparing a known cell's features to a specific cell's feature in order to classify the cell, would have been obvious to attempt using the feature of spatial frequency of a known cell to classify other cells, due to it's well known success in classification based on features. The modification

to the combination of Nicolette and Basiji, could be accomplished by including a template matching algorithm, which allows the comparison of a template, previous collected data of a known type (template), to newly acquired image data to identify the individual cell. Further a person of ordinary skill in the art would have recognized the compatibility of template matching with the method of the combination of Nicolette and Basiji. The combination has a reasonable expectation of success in that the modifications can be made using conventional and well known engineering and/or programming techniques, the template matching as taught by Young is not altered and continues to perform the same function as separately, and the resultant combination produces the highly predictable result of "comparing the spatial frequency content of the side scatter image of the specific cell to the spatial frequency content data of the side scatter image of the known cell type to determine if the specific cell corresponds to the known cell type."

As to Claim 30, the combination of Nicoletti, Basiji and Young teach the method of claim 29 wherein the step of analyzing determines that the specific cell is a viable cell, when: the image data indicates that the nuclear marker has not crossed the cellular membrane (***Nicoletti, page 4, ¶2-3, page 5, ¶1. "Whereas the DNA content of unfixed apoptotic cells is unchanged compared to normal (viable) cells", "the difference in the DNA fluorescence of normal (viable) and apoptotic cells is minimal or undetectable" page3, ¶1-2, thus in view of the reduced stainability of apoptotic cells, one concludes the viable cells also have a reduced stainability, thus the nuclear marker has not crossed the cellular membrane***); and the spatial frequency content of the side scatter image of the specific cell corresponds to a previously

determined spatial frequency content of a side scatter image of a viable cell (***template matching of spatial frequencies as taught by the combination***).

As to Claim 31, the combination of Nicoletti, Basiji and Young teach the method of claim 29 wherein the step of analyzing determines that the specific cell is a viable cell, when: the image data indicates that the nuclear marker has not crossed the cellular membrane (***Nicoletti, page 4, ¶2-3, page 5, ¶1, "Whereas the DNA content of unfixed apoptotic cells is unchanged compared to normal (viable) cells", "the difference in the DNA fluorescence of normal (viable) and apoptotic cells is minimal or undetectable" page3, ¶1-2, thus in view of the reduced stainability of apoptotic cells, one concludes the viable cells also have a reduced stainability, thus the nuclear marker has not crossed the cellular membrane***); and the spatial frequency content of the side scatter image of the specific cell does not correspond to a previously determined spatial frequency content of a side scatter image of an early apoptotic cell (***Nicoletti, page 4, ¶2-3, page 5, ¶1, further in view of the teachings of Nicoletti there are measurable morphological and physical properties (cells can be distinguished based on cell sizes see cells undergoing apoptosis exhibit cell shrinkage, condensation of chromatin and cytoplasm, so a viable cell what have a larger cellular area than say cells undergoing apoptosis – examiner believes this would be easy determined in the spatial frequency content for distinguishing viable verse early apoptotic***)

As to Claim 33, the combination of Nicoletti, Basiji and Young teach the method of claim 29 wherein the step of analyzing determines that the specific cell is a cell in an early apoptotic cell

when: the image data indicates that the nuclear marker has not crossed the cellular membrane (***Nicoletti, page 2, ¶1, page 3, ¶4, page 4, ¶1-2, one would expect to determine an early stage of apoptosis based on the teachings of reduced stainability of apoptotic cells (Applicant's the nuclear marker has not crossed the cellular membrane) is the direct consequence of partial loss of DNA due to the activation of endogenous nucleases and diffusion of low-molecular weight DNA outside the cells"***); and the spatial frequency content of the side scatter image of the specific cell does not correspond to a previously determined spatial frequency content of a side scatter image of a viable cell (***Nicoletti, page 4, ¶2-3, page 5, ¶1, further in view of the teachings of Nicoletti there are measurable morphological and physical properties (cells can be distinguished based on cell sizes see cells undergoing apoptosis exhibit cell shrinkage, condensation of chromatin and cytoplasm, so a viable cell what have a larger cellular area than say cells undergoing apoptosis – examiner believes this would be easy determined in the spatial frequency content for distinguishing viable verse early apoptotic***).

As to Claim 34, the combination of Nicoletti, Basiji and Young teach the method of claim 29 wherein the step of analyzing determines that the specific cell is a cell in an early apoptotic cell when: the image data indicates that the nuclear marker has not crossed the cellular membrane (***Nicoletti, page 2, ¶1, page 3, ¶4, page 4, ¶1-2, one would expect to determine an early stage of apoptosis based on the teachings of reduced stainability of apoptotic cells (Applicant's the nuclear marker has not crossed the cellular membrane) is the direct consequence of partial loss of DNA due to the activation of endogenous nucleases and diffusion of low-molecular weight DNA outside the cells"***); and the spatial frequency content of

the side scatter image of the specific cell corresponds to a previously determined spatial frequency content of a side scatter image of an early apoptotic cell (***template matching of spatial frequencies as taught by the combination***).

As to Claim 36, the combination of Nicoletti, Basiji and Young teach the method of claim 29 wherein the step of analyzing determines that the specific cell is a late apoptotic cell when: the image data indicates that the nuclear marker has crossed the cellular membrane (***Nicoletti, page 2, ¶1-3, given the emerging secondary necrosis of later stages of apoptosis (late apoptotic cell), one skilled in the art would expect to find traces of stained cellular DNA, given that necrotic cells take on stain (the nuclear marker has crossed the cellular membrane) (In later stages of apoptosis the intensity of both FSC and SSC decreases due to the emerging secondary necrosis)***); and the spatial frequency content of the side scatter image of the specific cell corresponds to a previously determined spatial frequency content of a side scatter image of a late apoptotic cell (***template matching of spatial frequencies as taught by the combination***).

As to Claim 37, the combination of Nicoletti, Basiji and Young teach the method of claim 29 wherein the step of analyzing determines that the specific cell is a late apoptotic cell when: the image data indicates that the nuclear marker has crossed the cellular membrane (***Nicoletti, page 2, ¶1-3, given the emerging secondary necrosis of later stages of apoptosis (late apoptotic cell), one skilled in the art would expect to find traces of stained cellular DNA, given that necrotic cells take on stain (the nuclear marker has crossed the cellular membrane) (In later stages of apoptosis the intensity of both FSC and SSC decreases due to the emerging secondary necrosis)***); and the spatial frequency content of the side scatter image of the specific

cell does not correspond to a previously determined spatial frequency content of a side scatter image of a necrotic cell (***Nicoletti, page 2, ¶1-3, in necrotic cells the cellular size would increase in view of rupture of plasma membrane and leakage of cell content, which would be distinguishable in the spatial frequency from a a late apoptotic cell whose size is smaller, see (cell shrinkage, condensation of chromatin and cytoplasm)***).

As to Claim 39, the combination of Nicoletti, Basiji and Young teach the method of claim 29 wherein the step of analyzing determines that the specific cell is a necrotic cell when: the image data indicates that the nuclear marker has crossed the cellular membrane (***Nicoletti, page 2, ¶2-3, necrotic cells take on stain (marker crossed the cellular membrane) further the teachings discloses measurable morphological and physical properties, cellular size would increase in view of rupture of plasma membrane and leakage of cell content, which would be observed by the spatial frequency content of the image***); and the spatial frequency content of the side scatter image of the specific cell does not correspond to a previously determined spatial frequency content of a side scatter image of a late apoptotic cell (***Nicoletti, page 2, ¶1-3, in necrotic cells the cellular size would increase in view of rupture of plasma membrane and leakage of cell content, which would be distinguishable in the spatial frequency from a a late apoptotic cell whose size is smaller, see (cell shrinkage, condensation of chromatin and cytoplasm)***).

As to Claim 40, the combination of Nicoletti, Basiji and Young teach the method of claim 29 wherein the step of analyzing determines that the specific cell is a necrotic cell when: the image data indicates that the nuclear marker has crossed the cellular membrane (***Nicoletti, page 2, ¶2-3, (marker crossed the cellular membrane) necrotic cells take on stain further the***

teachings discloses measurable morphological and physical properties, cellular size would increase in view of rupture of plasma membrane and leakage of cell content, which would be observed by the spatial frequency content of the image); and the spatial frequency content of the side scatter image of the specific cell corresponds to a previously determined spatial frequency content of a side scatter image of a necrotic cell (***template matching of spatial frequencies as taught by the combination***).

B.) Claims 8-23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nicoletti in view of Ortyn et al. ("Ortyn") [US PGPub #2002/0071121] and further in view of Young

As to Claim 8, Nicoletti outlines the problem to be solved (identifying a specific cell), and discloses a method for identifying a specific cell, to enable a determination to be made as to whether the specific cell corresponds to a known cell type, wherein the known cell type relates to the viability of the cell, (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, distinguish between late state apoptotic cells, necrotic cells, and viable cells***), comprising: providing content data from a brightfield image of the known cell type, and comparing the content to determine the cell type (***Nicoletti, Fig. 2, see page 2, section a., Physical parameters of apoptotic cells ¶1-2, page 3, section b. DNA content analysis ¶1,, page 4, ¶1 - 2, teaches common methods that exploit physical parameters (cell size, granularity, cell density) and DNA content analysis (stainability) of cells to determine their viability, using combination of light scattered in the forward direction (brightfield) and a lateral direction (side scatter image)***). Further, Nicoletti discloses regardless of the flow cytometry technique used to measure apoptosis, in most situations the type of cell death should be confirmed by direct microscope inspection. Apoptotic cells display a very specific pattern of morphological changes at the light,

electron and fluorescence microscope and this should be the deciding factor when ambiguity arises regarding the mechanism of cell death (***Nicoletti, Fig. 2, see page 2, section a., Physical parameters of apoptotic cells ¶1-2, page 3, section b. DNA content analysis ¶1,, page 4, ¶1 - 2, teaches common methods that exploit physical parameters (cell size, granularity, cell density) and DNA content analysis (stainability) of cells to determine their viability***). However Nicoletti silent to collecting spatial frequency of a specific cell, and comparing spatial frequency of a know cell type to the collected spatial frequency of the specific cell to determine if specific cell corresponds to the known cell type.

Enter Ortyn, who teaches a system that performs a method for collecting features for examining a specific cell, comprising:

directing incident light at a cell (***Ortyn, [0029], [0030], teaches having a light source incident upon the object (cell)***), using a detector to obtain the brightfield image of the specific cell (***Ortyn, [0124], [0071]***). Collecting spatial frequency content data of an individual cell from a brightfield image (***Ortyn, [0124], [0064]***). Furthermore, Ortyn teaches using spatial frequency content to be used in cell analysis, and further suggests that a cell can be identified using the morphological parameters (spatial frequency content) (***Ortyn, [0064]***).

One skill in the art would have been motivated to combine the teachings of Nicoletti to the apparatus of Ortyn. Ortyn's appartus is an improvement over a basic flow cytomerty, as it offers considerable advantages over systems employed for cell and particle analysis in the prior art, specifically the ability to combine an optical dispersion system with a TDI detector that produces an output signal in response to the images of cells and other objects that are directed on the TDI detector (***Ortyn, [0063]***). Ortyn's apparatus allows imaging of multiple objects on the

TDI detector at the same time, and each object in the image can be spectrally decomposed to discriminate object features by absorption, scatter, reflection or probe emissions using a common TDI detector for analysis (***Ortyn, [0063]***). Ortyn's apparatus solves the caveat disclosed by Nicolette "most situations the type of cell death should be confirmed by direct microscope inspection", as the TDI detector can replace the requirement for direct microscope inspection, as the TDI detector can achieve a desired resolution (optical magnification).

However, the combination of Nicolette and Ortyn are silent to a determining as to whether the specific cell corresponds to a known cell type, comprising the steps of: providing spatial frequency content data from a side scatter image of the known cell type; and comparing the spatial frequency content of the side scatter image of the specific cell to the spatial frequency content data of the side scatter image of the known cell type to determine if the specific cell corresponds to the known cell type.

Enter Young, who discloses a general method for cell identification based on template matching (cell comparison), where the templates are constructed from a known cell types (***Young, abstract, page 192, section 4. discussion***). Further, Young motivates this method by demonstrated its ability to automatically identify and measure individual cells in clusters (***Young, abstract***). Young's "Template matching" is a method for automating the identification and measuring of cells in microscope image, where correspondences are found between a template sub-image and the full image, based on a goodness-of-fit statistic evaluated at all possible positions, where Young utilizes FFTs in order to reduce computer time to an acceptable level (***Young, abstract, page 187, left col., paragraph 3***).

One skilled in the art would have recognized that this technique of template matching, the comparing a known cell's features to a specific cell's feature in order to classify the cell, would have been obvious to attempt using the feature of spatial frequency of a known cell to classify other cells, due to it's well known success in classification based on features. The modification to the combination of Nicolette and Ortyn, could be accomplished by including a template matching algorithm, which allows the comparison of a template, previous collected data of a known type (template), to newly acquired image data to identify the individual cell. Further a person of ordinary skill in the art would have recognized the compatibility of template matching with the method of the combination of Nicolette and Ortyn. The combination has a reasonable expectation of success in that the modifications can be made using conventional and well known engineering and/or programming techniques, the template matching as taught by Young is not altered and continues to perform the same function as separately, and the resultant combination produces the highly predictable result of "comparing the spatial frequency content of the brightfield image of the specific cell to the spatial frequency content data of the brightfield image of the known cell type to determine if the specific cell corresponds to the known cell type."

As to Claim 9, the combination of Nicoletti, Ortyn and Young teach the method of claim 8 wherein there is relative motion between the specific cell and the detector (***Ortyn, [0014], [0017], [0018], the detector captures the velocity (relative motion) between the cells and the detector***).

As to Claim 10, the combination of Nicoletti, Ortyn and Young teach the method of claim 8 wherein the specific cell identified is contained within a heterogeneous cell population, and brightfield image data is collected for the heterogeneous cell population (***Ortyn, Abstract,***

[0010]) (***Nicoletti, see page 3, section a. Physical parameters of apoptotic cells, ¶4, "to identify the phenotype of apoptotic cells in a heterogeneous cell population"***).

As to Claim 11, the combination of Nicoletti, Ortyn and Young teach the method of claim 8 wherein the specific cell identified is an apoptotic cell (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, distinguish "apoptotic cells"***).

As to Claim 12, the combination of Nicoletti, Ortyn and Young teach the method of claim 11 wherein the apoptotic cell is an early stage apoptotic cell ***or a late stage apoptotic cell*** (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, distinguish between late state apoptotic cells***)

As to Claim 13, the combination of Nicoletti, Ortyn and Young teach the method of claim 8 wherein the specific cell identified is a necrotic cell (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, distinguish "necrotic cells"***)

As to Claim 14, the combination of Nicoletti, Ortyn and Young teach the method of claim 8 wherein the specific cell identified is at least one of an apoptotic cell and a necrotic cell (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, distinguish between late state apoptotic cells, necrotic cells, and viable cells***).

As to Claim 15, the combination of Nicoletti, Ortyn and Young teach the method of claim 8 wherein the spatial frequency content is of the nucleus (***Ortyn, Abstract, [0064], measures spatial frequency of the nuclear area (nucleus)***) (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, a light scatter in a forward direction (brightfield) correlates with cell size (nuclear area)***).

As to Claim 16, Nicoletti outlines the problem to be solved (identifying a specific cell), and discloses a method for identifying a specific cell, to enable a determination to be made as to whether the specific cell corresponds to a known cell type, wherein the known cell type relates to the viability of the cell, (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, distinguish between late state apoptotic cells, necrotic cells, and viable cells***), comprising: providing content data from a image of the known cell type hat has been marked with a single nuclear marker, contacting the specific cell with the nuclear marker, and comparing the content to determine the cell type (***Nicoletti, Fig. 3, see page 4, ¶4, page 5, ¶1 teaches staining with PI or DAPI stain help identify a cell, see Figure 3 which compares the scatter analysis of an image with a single fluorescence – which helps identify apoptotic nuclei***). Further, Nicoletti discloses regardless of the flow cytometry technique used to measure apoptosis, in most situations the type of cell death should be confirmed by direct microscope inspection. Apoptotic cells display a very specific pattern of morphological changes at the light, electron and fluorescence microscope and this should be the deciding factor when ambiguity arises regarding the mechanism of cell death (***Nicoletti, Fig. 2, see page 2, section a., Physical parameters of apoptotic cells ¶1-2, page 3, section b. DNA content analysis ¶1., page 4, ¶1 - 2, teaches common methods that exploit physical parameters (cell size, granularity, cell density) and DNA content analysis (stainability) of cells to determine their viability***). However Nicoletti silent to collecting spatial frequency of a specific cell, and comparing spatial frequency of a know cell type to the collected spatial frequency of the specific cell to determine if specific cell corresponds to the known cell type.

Enter Ortyn, who teaches a system that performs a method for collecting features for examining a specific cell, comprising:

contacting a cell with a nuclear marker (***Ortyn, [0010], characterization of numerous [fluorescent markers]***), using a directing incident light at the marked cell (***Ortyn, [0029]***) using a detector to obtain an image of the cell (***Ortyn, [0124], [0071]***). Furthermore, Ortyn teaches using spatial frequency content to be used in cell analysis, and further suggests that a cell can be identified using the morphological parameters (spatial frequency content) (***Ortyn, [0124], [0064]***).

One skill in the art would have been motivated to combine the teachings of Nicoletti to the apparatus of Ortyn. Ortyn's appartus is an improvement over a basic flow cytomerty, as it offers considerable advantages over systems employed for cell and particle analysis in the prior art, specifically the ability to combine an optical dispersion system with a TDI detector that produces an output signal in response to the images of cells and other objects that are directed on the TDI detector (***Ortyn, [0063]***). Ortyn's apparatus allows imaging of multiple objects on the TDI detector at the same time, and each object in the image can be spectrally decomposed to discriminate object features by absorption, scatter, reflection or probe emissions using a common TDI detector for analysis (***Ortyn, [0063]***). Ortyn's apparatus solves the caveat disclosed by Nicolette "most situations the type of cell death should be confirmed by direct microscope inspection", as the TDI detector can replace the requirement for direct microscope inspection, as the TDI detector can achieve a desired resolution (optical magnification).

However, the combination of Nicolette and Ortyn are silent to a determining as to whether the specific cell corresponds to a known cell type, comprising the steps of: providing an

image of the known cell type that has been marked with a nuclear marker; providing spatial frequency content data from the image of the known cell type that has been marked with the nuclear marker, comparing the image of the marked specific cell and in combination a spatial frequency content of the image of the marked specific cell to identify a to the marked image of the known cell and the spatial frequency content of the marked image of the known cell type to determine if the specific cell corresponds to the known cell type.

Enter Young, who discloses a general method for cell identification based on template matching (cell comparison), where the templates are constructed from a known cell types (***Young, abstract, page 192, section 4. discussion***). Further, Young motivates this method by demonstrated its ability to automatically identify and measure individual cells in clusters (***Young, abstract***). Young's "Template matching" is a method for automating the identification and measuring of cells in microscope image, where correspondences are found between a template sub-image and the full image, based on a goodness-of-fit statistic evaluated at all possible positions, where Young utilizes FFTs in order to reduce computer time to an acceptable level (***Young, abstract, page 187, left col., paragraph 3***).

One skilled in the art would have recognized that this technique of template matching, the comparing a known cell's features to a specific cell's feature in order to classify the cell, would have been obvious to attempt using the feature of spatial frequency of a known cell to classify other cells, due to it's well known success in classification based on features. The modification to the combination of Nicolette and Ortyn, could be accomplished by including a template matching algorithm, which allows the comparison of a template, previous collected data of a known type (template), to newly acquired image data to identify the individual cell. Further a

person of ordinary skill in the art would have recognized the compatibility of template matching with the method of the combination of Nicolette and Ortyn. The combination has a reasonable expectation of success in that the modifications can be made using conventional and well known engineering and/or programming techniques, the template matching as taught by Young is not altered and continues to perform the same function as separately, and the resultant combination produces the highly predictable result of "comparing the image of the marked specific cell and in combination a spatial frequency content of the image of the marked specific cell to identify a to the marked image of the known cell and the spatial frequency content of the marked image of the known cell type to determine if the specific cell corresponds to the known cell type."

As to Claim 17, the combination of Nicoletti, Ortyn and Young teach the method of claim 16 wherein there is relative motion between the specific cell and the detector (***Ortyn, [0014], [0017], [0018], the detector captures the velocity (relative motion) between the cells and the detector***).

As to Claim 18, the combination of Nicoletti, Ortyn and Young teach the method of claim 16 wherein the specific cell identified is contained within a heterogeneous cell population, and image data is collected for the heterogeneous cell population (***Ortyn, Abstract, [0010]***) (***Nicoletti, see page 3, section a. Physical parameters of apoptotic cells, ¶4, "to identify the phenotype of apoptotic cells in a heterogeneous cell population"***).

As to Claim 19, the combination of Nicoletti, Ortyn and Young teach the method of claim 16 wherein the specific cell identified is an apoptotic cell (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, distinguish "apoptotic cells"***).

As to Claim 20, the combination of Nicoletti, Ortyn and Young teach the method of claim 19 wherein the apoptotic cell is an early stage apoptotic cell or a late stage apoptotic cell (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, distinguish between late state apoptotic cells***)

As to Claim 21, the combination of Nicoletti, Ortyn and Young teach the method of claim 16 wherein the specific cell identified is a necrotic cell (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, distinguish "necrotic cells"***)

As to Claim 22, the combination of Nicoletti, Ortyn and Young teach the method of claim 16 wherein the specific cell identified is at least one of an apoptotic cell and a necrotic cell (***Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2, distinguish between late state apoptotic cells, necrotic cells, and viable cells***).

As to Claim 23, the combination of Nicoletti, Ortyn and Young teach the method of claim 16 wherein a single nuclear marker is used the single nuclear marker and the spatial frequency content of the image enabling one to classify the specific cell as one of the following cell types:

a viable cell having a cellular membrane that is impermeable to the nuclear marker (***Nicoletti, page 4, ¶2-3, page 5, ¶1. "Whereas the DNA content of unfixed apoptotic cells is unchanged compared to normal cells", "the difference in the DNA fluorescence of normal***

and apoptotic cells is minimal or undetectable" page3, ¶1-2, thus in view of the reduced stainability of apoptotic cells, one concludes the viable cells also have a reduced stainability(impermeable), further in view of the teachings of Nicoletti there are measurable morphological and physical properties (cells can be distinguished based on cell sizes see cells undergoing apoptosis exhibit cell shrinkage, condensation of chromatin and cytoplasm and necrotic death of a cell is characterized by a reduction in both FSC and SSC (probably due to a rupture of plasma membrane and leakage of the cell's content), so a viable cell what have a larger cellular area than say cells undergoing apoptosis – and visible in the FSC (brightfield) would displays the cell size);

a cell in an early stage of apoptosis and which has a cellular membrane that is impermeable to the nuclear marker (***Nicoletti, page 2, ¶1, page 3, ¶4, page 4, ¶1-2, one would expect to determine an early stage of apoptosis based on the teachings of reduced stainability of apoptotic cells (Applicant's impermeable to the nuclear marker) is the direct consequence of partial loss of DNA due to the activation of endogenous nucleases and diffusion of low-molecular weight DNA outside the cells", further the teachings discloses measurable morphological and physical properties (cell shrinkage, condensation of chromatin and cytoplasm), which would be observed by the spatial frequency content of the image***);

a cell in a late stage of apoptosis and which has a cellular membrane that is permeable to the nuclear marker (***Nicoletti, page 2, ¶1-3, given the emerging secondary necrosis of later stages of apoptosis, one skilled in the art would expect to find traces of stained cellular DNA, given that necrotic cells take on stain (In later stages of apoptosis the intensity of both FSC and SSC decreases due to the emerging secondary necrosis), further the teachings discloses***

measurable morphological and physical properties (cell shrinkage, condensation of chromatin and cytoplasm), which would be observed by the spatial frequency content of the image); and

a necrotic cell which has a cellular membrane that is permeable to the nuclear marker (***Nicoletti, page 2, ¶2-3, necrotic cells take on stain further the teachings discloses measurable morphological and physical properties, cellular size would increase in view of rupture of plasma membrane and leakage of cell content,, which would be observed by the spatial frequency content of the image***).

C.) Claim 24 is rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Nicoletti, Ortyn and Young as applied to claim 16 above, and further in view of Fraatz (US Patent # 5372936, hereinafter Fraatz).

As to Claim 24, the combination of Nicoletti, Ortyn and Young teach the method of claim 16. However, the combination of Nicoletti, Ortyn and Young doesn't explicitly teach wherein the single nuclear marker is 7-aminoactinomycin D.

Fraatz teaches using 7-aminoactinomycin D as a maker for imagining samples (**Fraatz, Column 8, Table 1, Table 2, Column 6, lines 1-20**). Fraatz performs analysis for identifying biological activities in specimens (cells). It would have been obvious to one of ordinary skilled in the art at the time of inventions to modify the method for identifying cells of the combination of Ortyn and Young, by using 7-aminoactinomycin D as the nuclear marker as to the teaching of Fraatz. The combination of Nicoletti, Ortyn and Fraatz are analogous in the art of image based biological analysis. One of ordinary skilled in the art would have been motivated to combine the teachings of Fraatz to the method of the combination of Nicoletti, Ortyn and Young in order to

use the nuclear marker, 7-aminoactinomycin D, since it has useful properties (fluorescent dye) that would enable the isolation of cells in the image, as taught by Fraatz.

Further, the combination of the combination of Nicoletti, Ortyn and Young and Fraatz collectively teach all of the claimed elements, the teaching of Fraatz performs the same function in combination with the combination of Nicoletti, Ortyn and Young as taught individually in Fraatz, and the results would be highly predictable (Identifying cell in the image using the fluorescent dye (7-aminoactinomycin D) as a nuclear maker).

Allowable Subject Matter

1. Claims 45-48 would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims and removing any 112 2nd issues.

As to Claim 45 and 48, the claims are not rejected because the following limitation is not taught in the prior art: "wherein the spatial frequency content of the side scatter image is calculated by computing a standard deviation of individual pixel intensities within the image"

Note to applicant, Claim 45 and 48 are identical.

As to Claim 46, the claim is not rejected because the following limitation is not taught in the prior art: "wherein the spatial frequency content of the brightfield image is calculated by computing a standard deviation of individual pixel intensities within the image"

As to Claim 47, the claim is not rejected because the following limitation is not taught in the prior art: "wherein the spatial frequency content of the image is calculated by computing a standard deviation of individual pixel intensities within the image"

Comment on 35 USC § 101

Independent claim 1, 8, 16, and 29 are in a "process" claim format and have been analyzed in light of *Bilski et al v. Kappos* [1], and the relevant guidance [2], [3]. The independent claims are not directed to an abstract idea at least because the independent claims encompasses more than just a statement of concept, and describes a particular solution to identifying cells using spatial frequency. Furthermore, the independent claim tangibly implements the method at least because a processor or equivalent hardware is necessary to perform the claimed "collecting/providing" and "analyzing/comparing" steps. Therefore, based upon consideration of all the relevant factors[3] with respect to the claims as a whole, claims 1, 8, 16, and 29 are not directed to an abstract idea.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jason Heidemann:

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[1] *See Bilski et al v. Kappos (SCt. 08-964),*

[2] *See Memorandum to the Examining Corps, Regarding the Supreme Court Decision in Bilski v. Kappos, issued June 28, 2010, available at http://www.uspto.gov/patents/law/exam/memoranda.jsp*

[3] *See Interim Guidance for Determining Subject Matter Eligibility for Process Claims in View of Bilski v. Kappos, Federal Registrar, Vol. 75, No. 143, issued July 27, 201*

- phone (571) 270-5173,
- fax (571) 270-6173, or
- e-mail jason.heidemann@uspto.gov.

The examiner can normally be reached on Monday - Thursday/7:30 A.M. to 5:00 P.M.. For e-mail communications, please note MPEP 502.03, which states, in relevant part, "[w]ithout a written authorization by applicant in place, the USPTO will not respond via Internet e-mail to any Internet correspondence which contains information subject to the confidentiality requirement as set forth in 35 U.S.C. § 122." A sample authorization form which may be used by applicant can be found in MPEP 502.03 section II.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Matthew Bella can be reached on 571-272-7778. The fax phone numbers for the organization where this application or proceeding is assigned are 571-273-8300 for regular communications and 571-273-8300 for After Final communications. TC 2600's customer service number is 571-272-2600.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Jason Heidemann/ 07/07/2011
Examiner, Art Unit 2624

/Sath V. Perungavoor/
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Primary Examiner, Art Unit 2624
Dated: July 12, 2011